Congenital cytomegalovirus infection registry in Flanders : opportunities and pitfalls

Congenital Cytomegalovirus Infection Registry in Flanders : Opportunities and Pitfalls.

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Abstract

In 2007, a prospective multicentre registry was set up to collect data on incidence and outcome of children with congenital cytomegalovirus infection in Flanders. A consensus was reached about management and follow up of cytomegalovirus-infected children. With this registration we aimed at gathering information on congenital cytomegalovirus infection in Flanders and evaluating the consensus on management and therapy. Children with proven congenital cytomegalovirus infection were eligible for registration in the database. Information on prenatal and neonatal management, therapy and follow up until 6 years was obtained. Between 2007 and 2017, 686 children were registered. Data on the prenatal and neonatal characteristics in children with congenital cytomegalovirus infection are reported.

Conclusion

In this article we report on our experience of conducting a registry for cCMV in Flanders. Eleven years of collecting data on CMV in a multicenter setting has shown us some pitfalls and opportunities. We address some of the problems and aim at improving our data gathering. We encourage other groups to share their data. Better knowledge of the burden of the disease will be important to guide future management strategies.

Keywords

congenital infection, congenital cytomegalovirus infection, registry, database, strengths, limitations

Funding details

Funding: There is no funding source.

Disclosure statement

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

Informed consent: Informed consent was obtained from all individual participants included in the study.

Introduction

Congenital cytomegalovirus (cCMV) infection is the most common congenital infection worldwide with a reported prevalence in the developed world being approximately 7 per 1000 births. [1-6] Congenital CMV has a significant long-term impact on affected children, being the major cause of non-hereditary sensorineural hearing loss and major infectious cause of neurodevelopmental abnormalities in infants born in developed countries. [7,8] Long-term sequelae are more common in symptomatic children (approximately 50%) but they are also found in around 13% of the asymptomatic children. [9] Despite this important disease burden, cCMV remains largely unrecognized due to lack of systematic screening. Moreover, there is limited evidence on which to base management and therapy of children with cCMV infection. Recently, 2 research groups published their consensus statement and recommendations on diagnosis and management. [5,6] Both groups emphasize the importance of establishing a uniform definition of symptomatic/asymptomatic disease and the need for collecting accurate data on diagnosis, clinical signs, additional investigations and therapy in children with cCMV.

In 2006, the Flemish Society of Pediatrics' Neonatology and Perinatal Epidemiology Working Group founded a working group for cCMV infection, consisting of neonatologists, pediatricians and otorhinolaryngologists. The working group reached a consensus on about systematic diagnosis, follow up and indications for treatment for children with cCMV infection [9]. In 2007 the working group started the systematic registration of diagnosis, follow up and treatment of patients that presented with cCMV in the 6 collaborating hospitals. An online registry was made available since 2013.

In this article, we want to describe our experience with conducting a registry by highlighting the strengths and addressing the limitations.

Subjects and methods

Data collection

Six centers in Flanders, Belgium (Ghent University hospital, University hospital Leuven, University hospital Antwerp, Middelheim Antwerp, Hospital Network Antwerp and AZ Sint Jan Bruges) initially participated in the registration. In the 11-year period 2 other hospitals joined this multicenter registration (ZOL Genk, AZ Turnhout). The registration was approved by the ethics committee and was enlisted at the privacy commission. Children were included in the register only after written informed consent of the parents or legal guardians. Where possible, missing data were completed by searching the medical files. Some data remained 'unknown' or 'missing'.

Patients and methods

Patients were included in the registry only after a confirmed diagnosis of cCMV. Diagnosis in the neonatal period was made by viral isolation and/or PCR on urine taken within the first 3 weeks of life. Over the last 3 years, viral culture of saliva has also been used as diagnostic tool. Retrospective diagnosis (after age of 21 days) was made by PCR on dried blood spot (DBS). In Belgium, this card is kept in store for 5 years.

Definition symptomatic/asymptomatic

Following the Flemish consensus, neonates were classified as 'symptomatic' if additional investigations revealed at least one of the following : at least 2 or more

clinical findings at birth suggestive of cCMV (microcephaly, seizures, intra-uterine growth restriction (IUGR), petechiae, thrombocytopenia, hepatomegaly, splenomegaly), hearing loss, chorioretinitis or lesions on central nervous system imaging (except pseudocysts or isolated striatal vasculopathy). Patients with late diagnosis of cCMV (e.g. because of delayed onset of hearing loss) were classified as asymptomatic, since there had been no reason to screen for cCMV at birth.

Data

At enrolment, data are collected on the prenatal period (timing of seroconversion, gravidity/mater and prenatal tests such as ultrasound, MRI, amniocenthesis), diagnosis (reason for testing, diagnostic tools, epidemiologic features), clinical features at birth, results of additional investigations (blood count, transaminases, CMV IgM/ IgG and CMV-DNA polymerase chain reaction (PCR) on serum, central nervous system imaging, audiological testing, ophthalmologic investigation) and therapy. All additional investigations were performed within 1 month after birth to identify those children eligible for treatment. Long-term data are collected on neuromotor skills, hearing and vision. For children with late diagnosis (> 21 days of age) these additional investigations have not been performed in most of the children.

Hearing

Possible methods for hearing evaluation are oto-acoustic emissions (OAE), automated auditory brainstem response with portable ALGO^{TM} or auditory brainstem evoked responses (ABR). Preference is given to ABR for hearing evaluation.

Central nervous system imaging

Central nervous system imaging was done by brain ultrasound, computed tomography scan (CT), magnetic resonance imaging (MRI) or a combination of these.

Ophthalmologic testing

Ophthalmologic evaluation was performed by fundoscopy.

Therapy

Indication for treatment was conform the consensus of the Flemish society of Pediatrics' Neonatology and Perinatal Epidemiology Working Group. Therapy is offered to all symptomatic children (unless bilateral severe neurosensory hearing loss). After being informed on the possible benefits and short and long term side-effects of antiviral therapy, parents are involved in the decision whether or not to start therapy. [9] Between 2007 and 2011, treatment consisted of intravenous ganciclovir during 6 weeks at a regimen of 6 mg/kg, twice daily. Since august 2011, oral treatment with valganciclovir during 6 weeks, 16 mg/kg twice daily, has been introduced. Since January 2017 we apply the 6 months regimen.

Follow-up

The follow-up differs slightly between symptomatic and asymptomatic children as is shown in figure 1. Data of audiological and neurodevelopmental follow-up are collected in the database up to the age of six years.

Results

Between January 2007 and July 2017, 686 children were included, of which 226 (32,9%) were classified as symptomatic and 460 (67,1%) as asymptomatic. An overview of the collected data is presented in table 1 (prenatal topics), table 2 (diagnostic tools and reasons for testing) and table 3 (results of the additional investigations). The detailed description of these results is beyond the scope of this article. We also presented the percentage of missing data on every topic, ranging from 5,5% tot 72, 5%, with one outlier of 93,7% for the topic 'prenatal MRI'.

The long-term hearing outcome of our patients has been published in 2015. [11] As for the long-term neurological outcome, we have insufficient data at this point to describe neurological outcome in an accurate manner.

Discussion

Congenital CMV is the most frequent congenital infection worldwide. The minority of the infected children are symptomatic at birth (10-15%) but long-term sequelae (neurodevelopmental delay, sensorineural hearing loss) can develop in both symptomatic and asymptomatic children and can have an important impact on child, parents and society. [1,2] For that reason, it is important to obtain data on congenital CMV infection as precisely as possible so pre- and postnatal management and therapy can be optimised. Large-scale screening programs could provide us with the data needed to fully know and understand the burden of cCMV and to develop recommendations on management of cCMV. However, those screening programs are not available at this point. The need for registration of data on cCMV has been addressed by several research groups.[2,4,6,12] The use of registries has become increasingly common and has led to enhancements in the understanding of many diseases.[13] Although the use of registries to describe a population or disease can have some opportunities, we must be aware of the possible pitfalls. There are some limitations inherent to the data collection process that can introduce bias and hence produce invalid results.

Our database (www.cmvreg.be) has registered children with cCMV since January 2007 in Flanders, Belgium. In this article we focus on the strengths and limitations of our database and make suggestions on how we can improve our data gathering.

Strenghts

From January 2007 until December 2017, 686 children were registered in our database. Children who were diagnosed at birth as well as children with late/retrospective diagnosis (i.e. > 28 days old) are included. We have established a cohort study which has given us a large dataset. This is one of the largest datasets of children with cCMV, collecting numerous topics of this disease from the prenatal period until the age of 6. This enables us to look for possible associations between risk factors and outcome and in this way helps us in decision-making, management and counselling. Many similarities between our data and previously described cCMV populations were found, which indicates that our population is representative for children with cCMV.

Changes over the years

Our registry started in 2007, over 10 years ago. The consensus on management and therapy of cCMV made by our working group, was based on literature and research from that time. Obviously, the former guidelines were adapted according to recent literature and new insights and hence our database forms. (e.g. the use of prenatal MRI, therapy regimen, choice of diagnostic tool)

In our population 32,9% of the children were classified as symptomatic, which is higher than found in literature. This can partly be explained by referral bias since symptomatic children are more likely to be referred to tertiary centers. Also, there is no universal screening so we do not have accurate data on the asymptomatic group as well, probably underestimating this percentage. Another explanation is the definition of symptomatic disease which differs between study groups. When reviewing the literature, variable definitions of symptomatic and asymptomatic cCMV infection are found across different studies. The earliest reports on cCMV infection define symptomatic cCMV infection as the triad of petechiae, hepatosplenomegaly and

jaundice. It was called ‘cytomegalic inclusion disease’. Children with severe infections also showed thrombocytopenic purpura, microcephaly, intrauterine growth restriction (IUGR) and chorioretinitis. The diagnosis was exclusively based on clinical symptoms and signs. With the advent of ultrasound, CT and MRI, anomalies seen on imaging such as intracranial calcifications, ventriculomegaly and white matter disease were increasingly incorporated in the definition. Today the most current definition of symptomatic cCMV infection is the presence of one or more of following symptoms: petechiae/rash, hepatosplenomegaly, jaundice, microcephaly, chorioretinitis and intracranial calcifications.[5,6] Significant IUGR, prematurity and seizures might be associated, but are aspecific for cCMV. Hearing loss is often not included in the definition.

Since 2000, universal newborn hearing screening is increasingly implemented in developed and developing countries. Congenital hearing loss is therefore diagnosed within weeks after birth. As screening for cCMV infection is part of the standard diagnostic protocol in children born with hearing loss, a large number of cCMV-infected children are diagnosed that way. We feel that a child with congenital hearing loss due to cCMV infection, even in the absence of other clinically apparent symptoms, should be considered a symptomatic child. In our database 55 children of the 226 symptomatic children (24%) were included because of isolated congenital hearing loss.

More recently, it is suggested that the symptomatic children should be classified as mild, moderate or severe symptomatic, since the traditional dichotomy between ‘apparent’ and ‘unapparent’ disease is becoming less meaningful. [5,6] It remains difficult to make this classification but as is suggested by Luck et al. it would be beneficial to develop a validated clinical scoring system for disease severity at

presentation and risk of sequelae. In this way, we could make classification more uniform worldwide.[5]

We agree with the consensus of ECCI (European Congenital CMV Initiative) that standardization of the definition of symptomatic and asymptomatic cCMV infection is important.[5] The actual heterogeneity in definitions limits direct comparison in literature of prevalence rates and risk figures of hearing loss in these groups. So in order to have a clear view on the burden of disease, to correctly counsel parents and to develop guidelines for follow-up and treatment of these children, it is essential that a uniform definition of symptomatic and asymptomatic cCMV infection is used. We should strive for a standardized definition adapted to modern diagnostic techniques. Hearing loss, as major sequel of the infection, should be part of the definition without exception.

As for diagnostic tools, viral isolation in urine was the golden standard in 2007, being the main diagnostic tool in our database so far. Since viral isolation in saliva and PCR (polymerase chain reaction) on urine have proven to be valuable alternative tools, we have altered this in our database and the consensus. [3,5,6,14]

Our results on CMV IgM and IgG reflect the findings in literature that there is no additional value of these tests [15]. For that reason, since 2018 performing IgM and IgG is no longer warranted for patients in our registry.

Since the introduction of the electronic database in 2013 we have added ‘gravidity’ and ‘mater’ to the database form. Most cCMV infections occur in neonates with older siblings. As is suggested in literature, preventive hygienic measures can lower the risk of seroconversion so it is important that parents are aware of this. By collecting data on this topic we hope to persuade the policymakers of the need of raising awareness of CMV in parents during pregnancy. [3,11,16]

Prenatal MRI has proven to be a useful examination and complementary to ultrasound. [17,18] So we have seen an increase in the use of prenatal MRI over the last 4 years. Hence we added this topic to the database. The majority of the data on prenatal MRI listed as 'missing' is from inclusions before this parameter was added. Therapy regimen changed over the years from 6 weeks intravenous Ganciclovir to 6 weeks oral Valganciclovir in 2011. Since January 2017 we apply the 6 months regimen of therapy with oral valganciclovir, according to worldwide consensus and recommendations. [5,6,19]

As for follow-up, since 2018 all children in Flanders with a confirmed neonatal hearing loss in Flanders will undergo a vestibular screening at the age of 6 months in the reference centers involved in the neonatal hearing screening program. Congebital CMV-related labyrinthitis can affect not only the auditory, but also the vestibular function. This in turn can have an impact on the motor development. Especially cCMV children with a congenital bilateral severe hearing loss are at risk for a vestibular dysfunction. [20] However vestibular dysfunction in cCMV can also be delayed in onset. Therefore we feel that in children with cCMV besides the hearing also the vestibular function and motor development should be followed longitudinally in the first years of life.

Limitations and pitfalls

We are aware of the fact that this registry has its limitations. The greatest threat to any study is bias. First, we encounter the problem of selection bias in our population. Inclusion of CMV-positive newborns depends on the goodwill and cooperation of pediatricians and otorhinolaryngologists in Flanders and is realised only after written informed consent of the parents. Children with clinical signs, hearing problems or

neurological impairment could be overrepresented in the registry, as symptomatic babies are more likely to be referred to tertiary centers, from where they are recruited. And since there is no universal screening for CMV at birth, we miss some of the asymptomatic children in our database if no reason for testing was present at birth. The fact that 8,6% of the children in our database are diagnosed at later age confirms the fact that not all children with cCMV are diagnosed or recognized. We also realise that the missing data on long-term follow up can be due to study drop out. The suggested follow up for our patients is not mandatory, so parents can decide at any moment not to attend follow up consultations. The questionnaire [21] we send out to all patients at age 6, will hopefully result in more complete data on neurologic development.

Secondly, we have information bias. As is seen in the results of the pre- and neonatal data we have missing data on all items. On some items, the proportion of missing data is quite high. This makes it difficult to perform multivariable analyses. It is not always clear whether data are missing due to the fact that the requested investigation was not performed or to the fact that the result is not known. Experts on cCMV worldwide aim to describe a consensus on diagnosis and management but they all admit that there are still some controversies. These controversies may lead to case-per-case or physician-per-physician variability in how a patient with cCMV is managed, resulting in missing data in a registry.[4,5,6] It is something to bear in mind when aiming for more elaborate/multinational registries.

Addressing the limitations

Eleven years of registrations has not only provided us with clinical data on our population but it also showed us the limitations of our registry. We have the

opportunity of addressing some of them in order to optimise our data gathering.

A major change in our data gathering was the introduction of the electronic version of the database. [22] The first 5 years of the registry, data were obtained by written forms that had to be filled in and sent to the database manager. As for follow-up results, the database manager had to distillate the needed data from reports from consultations which differed from center to center. When implementing the electronic database we made uniform forms on registration and follow-up, approved by all members of the Flanders working group. In this way, we are sure that al data are reported in a uniform manner which makes it easier to describe the results in our population. The electronic database is easily accessible for every pediatrician, otorhinolaryngolist or other physician that wants to enter a patient in the registry. [fig. 2] The database manager provides a personal login and password per user of the database. The users can always enter follow up data of their own patients but they can not see any data from other patients. In this way privacy of the patient is guaranteed. By making the database available online we aimed at recruiting more patients, also from non-tertiary hospitals. In the 11 years of registration, we have added 2 extra non-tertiary hospitals to our 6 collaborating hospitals. And numerous pediatricians from other hospitals have asked for a login to enter their patients. We hope this positive trend will continue. Although this electronic database makes the data registration easier, it still requires time investment of the physician.

To enhance the data recruitment of long-term follow up of our patients we have already taken some actions. All children are invited for a hearing evaluation at the age of six years. In this way we can have a final hearing evaluation in all children before ending the follow up. As for neurological follow-up, we send a questionnaire on neurological development for parents to fill in, to all patients at 6 years. It provides us

with information on the development of our patients, impaired or not. By this, we aim at gathering more complete data on neurological outcome in a larger group of patients and hope to correlate the described lesions on central imaging with neurodevelopmental outcomes.

Most of the international guidelines recommend follow-up until the age of six. [5,23,24] . As for the neurodevelopmental follow-up, this is a good timing. If developmental problems are expected, they will appear within the first years of life. As for the hearing screening, timing of follow-up remains a point of discussion. A review by Fletcher et al. has shown that studies with shorter follow-up (< 3 years) had lower rates of SNHL than studies where follow-up is performed beyond 5 years. In this review the median age at which delayed onset of hearing loss occurred varied widely, being 44 months in asymptomatic children en 33 months in symptomatic children. For progression of hearing loss the median age also varied considerably: 12-51 months in asymptomatic children and 26 months in symptomatic cCMV. All median ages being well under 6 years of age but most of the studies don't have longer follow-up. [25] Only one study in this review of 36 articles stated that delayed hearing loss or progression of hearing loss could occur as late as the mid-teens. [26] A study by Lanzieri et al. showed that the risk of developing SNHL in asymptomatic cCMV children after 5 years of age is the same as in uninfected children. Which might make the risk of missing severe SNHL beyond the age of six rather low. [27] However, this aspect of follow-up should be further investigated but for now international recommendations remain the same.

Raising awareness in healthcare professionals in Flanders for the Flemish consensus of guidelines on management practices remains important. The consensus made by the Flemish working group in 2018, is published on the website of the Flemish

Society of Pediatrics [28]. All pediatricians have free access to this consensus text. Continuous stimulating of physicians tot enter patients to the registry is necessary. At the end of our consensus text, we added a reminder for the database. But also on the reports of PCR or viral isolation on urine of our laboratory, a reminder to enter patients is added at the bottom of the page.

Many research groups advocate the development of international guidelines regarding uniform, evidence-based recommendations on the management of children with cCMV. These should be based on data of large-scale screening programs. [2,4,5,6] Since large-scale screening programs are not available at this point, we can only base our guidelines for consensus and management on data obtained by registries, like ours.

Conclusion

Conducting a registry is a dynamic process. We described the opportunities and pitfalls of a multicenter CMV registry in Flanders, Belgium during an eleven year period. We discussed the strengths and limitations of our database and have tried to address some of the problems in order to optimise data gathering. In this way we hope to recruit the majority of children with cCMV, described in a uniform manner, providing us with a less biased population of children with cCMV.

Worldwide, the systematic registration and follow up will become increasingly important to document the impact of forthcoming preventive and therapeutic measures. Both national as international collaboration is important. Therefore we aim at providing an easily accessible database that will lead to more complete recruitment and data on this population in Flanders and we hope to share this with international networks.

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Table 1. Prenatal characteristics of children in the registry.

n = number of children entered in the registry

Number of children in registry	**686**	
	n	**%**
Sex distribution		
Male	350	51%
Female	287	41,8%
Missing/unknown	49	7,2%
Gestational age at birth		
24 - 29+6 weeks	2	0,3%
30 – 36+6 weeks	53	7,7%
> 37 weeks	478	22,4%
Missing/unknown	152	22,4%
Gravidity		
1	79	11,5%
≥ 1	387	56,4%
Missing/unknown	220	32,1%
Mater		
0	85	12,4%
≥1	381	55,5%
Missing/unknown	220	32,1%
Time of seroconversion		
0-13 weeks	169	24,6%
14-27 weeks	142	20,7%
> 27 weeks	91	13,1%
Missing/unknown	284	41,4%
Amniocenthesis		
PCR positive	83	12,5%
PCR negative	32	4,6%

	Not performed	348	50,4%
	Missing/unknown	223	32,5%
Fetal ultrasound			
	Normal	440	64,1%
	Abnormal*	41	6%
	Not performed	4	0,6%
	Missing/unknown	201	29,3%
Prenatal MRI			
	Normal	25	3,6%
	Abnormal**	18	2,6%
	Missing/unknown	643	93,7%

*ascites, hydrocephaly, hyperechogenic bowel, oligohydramnios, IUGR, cysts, microcephaly, organomegaly

**ventriculomegaly, leuko-encephalitis, hepatosplenomegaly, cysts

Table 2. Reasons for testing for cCMV at birth and diagnostic tools used for testing. n = number of children entered in the registry.

Timing of diagnosis	**n**	**%**
≤21 days of age	541	78,9%
> 21 days of age	59	8,6%
Missing/unknown	86	12,5%
Reason for testing for cCMV		
Abnormal central imaging	18	2,6%
Dysmaturity	21	3,2%
Hearing loss	53	7,7%
Known maternal seroconversion	501	73%
Developmental delay	2	0,3%
Hematological disorders	16	2,3%
Other*	35	5%
Diagnostic tool used ≤ 21 days of age		
Viral isolation urine	483	
Viral isolation saliva	5	
PCR urine	108	
Diagnostic tool used > 21 days of age		
PCR on dried blood spot	80	
PCR urine	20	

*cholestasis, cataract, convulsions, hepatosplenomegaly, microcephaly

Table 3. Results of the additional investigations performed in children with cCMV.

n = number of children entered in the registry.

		n	%
Apparent clinical signs at birth ?			
	Yes	69	10,1%
	No	579	84,4%
	Missing/unknown	38	5,5%
Laboratory investigations			
CMV IgM			
	Positive	83	12,1%
	Negative	217	31,6%
	Missing	385	56,3%
CMV IgG			
	Positive	295	43%
	Negative	5	0,7%
	Missing	386	56,3%
White blood cell count			
	Normal	343	50%
	Leucopenia	2	0,3%
	Missing	341	49,7%
Thrombocytes count			
	Normal	301	43,9%
	Thrombocytopenia	39	5,7%
	Missing	346	50,4%
Liver enzymes (AST, ALT, gamma-GT)			
	Normal	198	28,9%
	Elevated	17	2,5%
	Missing	417	68,6%

Cranial ultrasound		
Normal	396	57,7%
Abnormal*	127	18,6%
Missing/not performed	162	23,7%
MRI		
Normal	306	44,6%
Abnormal**	98	14,3%
Missing/not performed	282	41,1%
Hearing evaluation at birth		
Bilateral hearing loss	49	7,1%
Unilateral hearing loss	62	9%
Normal	533	77,7%
Missing	42	6,2%
Ophthalmological evaluation		
Normal	597	87%
Chorioretinitis	4	0,5%
Missing/not performed	85	12,4%
Symptomatic cCMV ?		
Yes	226	32,9%
No	460	67,1%
Start therapy ?		
Yes, IV 6 weeks	29	4,2%
Yes, oral 6 weeks	65	9,5%
Yes, oral 6 months	4	0,6%

No	493	71,9%
Missing/unknown	95	13,8%

*cystic leukomalacia, ventricular adhesions, cysts, striatal vasculopathy, cystic germinolysis, calcifications, vermis hypoplasia

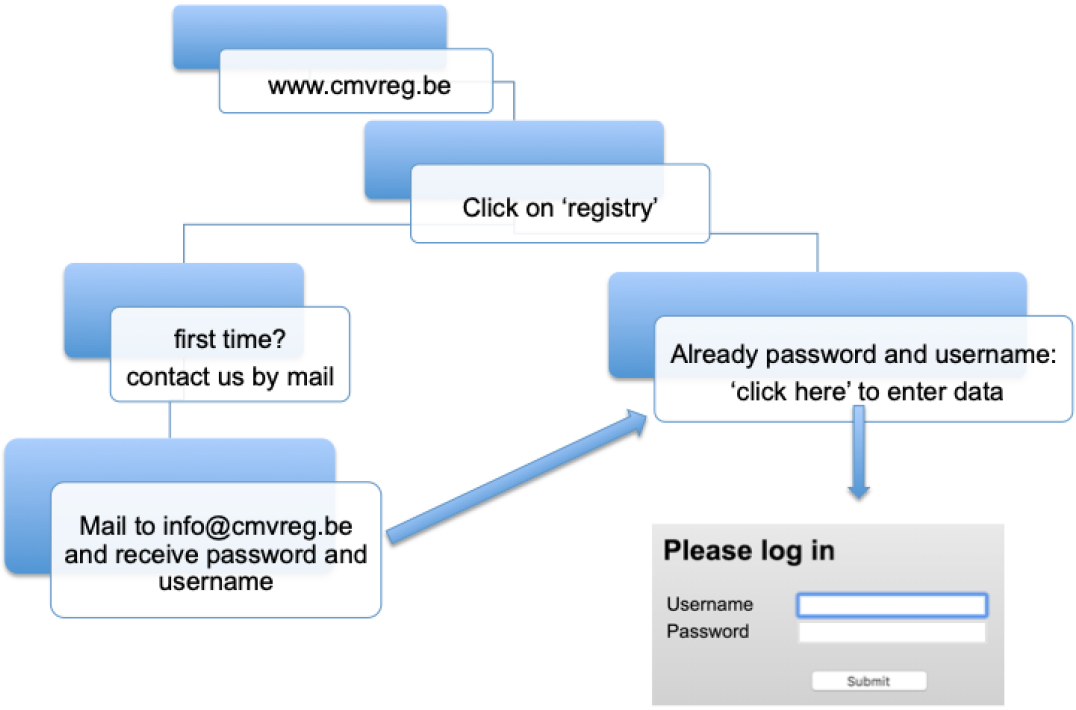
**Cystic leukomalacia, ventricular adhesions, cysts, gyration disorders, hyperintensity white matter, striatal vasculopathy,ventriculomegaly, vermis hypoplasia, polymicrogyria, leuko-encephalitis

Fig 1 Schematic follow-up of cCMV-patients in Flanders

	Birth	3-4 months	6 months	1yr	1,5yr	2yr	2,5yr	3yr	4yr	4,5yr	5 yr	6 yr
Vision	X1			X1		X1		X1	X1		X1	X1
Hearing	X2	X2	X2 (S)	X2	X2 (S)	X2	X2 (S)	X2	X2		X2	X2
Vestibular function			X2	X2		X2		X2	X2		X2	X2
Development		X3 (S)		X3	X3 (S)	X3 (IR)		X3 (IR)		X3(IR)		(Q)

X1 follow-up by ophthalmologist (funduscopy)
X2 follow-up by ENT-specialist (hearing screening en vestibular function)
X3 follow-up by center for developmental disorders
(S) only in symptomatic children
(IR) if required by specialists
(Q) by questionnaire send to parents

Fig 2. Access of the registry



	Birth	3–4 months	6 months	1yr	1,5yr	2yr	2,5yr	3yr	3,5yr	4yr	5 yr	6 yr
Vision	X1			X1		X1		X1		X1	X1	X1
Hearing	X2	X2	X2 (S)	X2	X2 (S)	X2	X2 (S)	X2		X2	X2	X2
Vestibular function			X2	X2		X2		X2		X2	X2	X2
Development		X3 (S)		X3	X3 (S)	X3 (IR)		X3 (IR)		X3 (IR)		(Q)

X1 follow-up by ophthalmologist (funduscopy)
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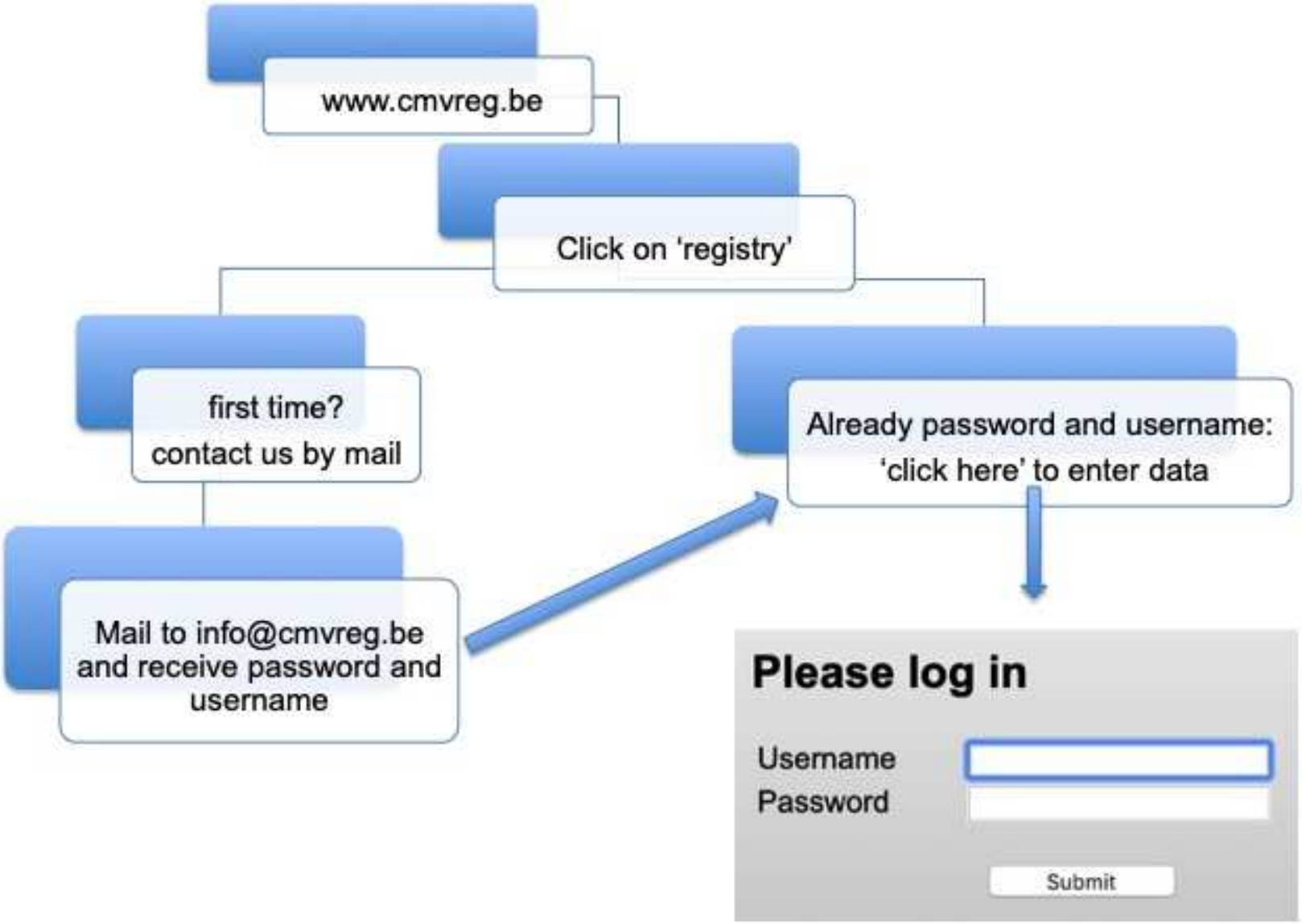
Figure 1. Schematic follow-up of cCMV patients in Flanders.



Dear editor,

Dear Professor Petrovic,

I have tried to answer all of the comments in the manuscript.

- Patients and methods and Table 2: in "Patients and methods" it is mentioned that diagnosis in the neonatal period was made in the first 3 weeks of life and retrospective diagnosis: after age of 28 days. "Table 2" mention < or > 28 days when addressing timing of diagnosis. This contradictions between both parts of the manuscript has to be resolved. Day 28 should also be added in one of the groups (neonatal period or retrospective diagnosis).

This was indeed a contradiction and needed to be changed to 21 days since wedon't use the same diagnostic tools before en after 21 days of age. I have changed it in both the text and the tables. I checked the figures but they didn't change.

- I would suggest to add schematic presentations of the performed follow-up as figure(s) to improve readability.

See fig 1.

- Changes over the years: please, comment how many children in the registry were included with isolated congenital hearing loss.

I added these data where it as asked.

- Limitations and pitfalls: please, include or at least refer to the source of the questionnaire send to the parents at the age of 6 years old.

I added a link to the questionnaire in the references.

Do you think that the final hearing and neurological evaluation at 6 years of age is sufficient? Please, discuss this issue in more detail.

I included a short piece of literature covering this subject in the text.

- As one of the aims of publication of this work would be the scientific promotion of the existing registry, I would suggest to add essential information to access and use the registry in a separate figure.

See figure 2. I focused on how to gain access to the registry. The use of the registry is straight forward and when the mail with username and password is send, a small manual to use the registry is send along.

- On extra, I added a short piece on a change in the follow-up we perform since 2018. Since then vestibular function is tested in all children with neonatal hearing loss, also in children with cCMV. I already added it to the manuscript under ‘changes over the years’ but if you don not agree since it was not in the original manuscript, then you can leave it out.

I hope these revisions will be sufficient,

Kind regards and thank you for reading my manuscript,

Annelies Keymeulen